# Validation of methodology for study the DNA-radiation interaction DNA.

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## Abstract

In the present work, experimental samples of DNA in aqueous solution irradiated with gamma rays were analyzed. The DNA fractions were fitted to different models and the results were compared with the ones reported in the literature. The results agree with similar studies according to the purity of the samples, indicating that the experimental method developed is reliable. Other irradiations with gamma, protons and neutrons are being accomplished, in order to verify a theoretical model, proposed by our group.

## Introduction

Our group has developed a method to accomplish this study. Many attempts have been made to obtain the optimized methodology which is present in another poster in this meeting. In this work an analysis of the first results of γ-irradiation was made and compared with other worker's results. Our objective is characterize the reliability of our experimental method.

## The Method

The method for the analysis of the DNA damage by radiation is described in another poster with the results data obtained.

## Data analysis

The data were fitted with some models to make the method validation.

### *Statistical Model*

Cowan (1987) developed a model for the breakage of DNA under de action of enzyme or radiation.

$$S=\frac{S(\mu,\phi)}{1-F(\mu,\phi,b)}\quad R=\frac{R(\mu,\phi,b)}{1-F(\mu,\phi,b)}\quad L=\frac{L(\mu,\phi,b)}{1-F(\mu,\phi,b)}$$

where $\mu=\mu_o+\lambda\cdot D$ is the number of hit (SSB) in the DNA, $\phi=\phi_o+\rho\cdot D$ is the numbers of cut (DSB) directly produced. Parameter *b* is the proportion of the zone where tow hits can produce a cut, $\lambda$ is the number of hit per dose unit and molecule and $\rho$ is the number of cut per dose unit and molecule produced directly.

Fitting all the equations at the same time, we get (figure 1):

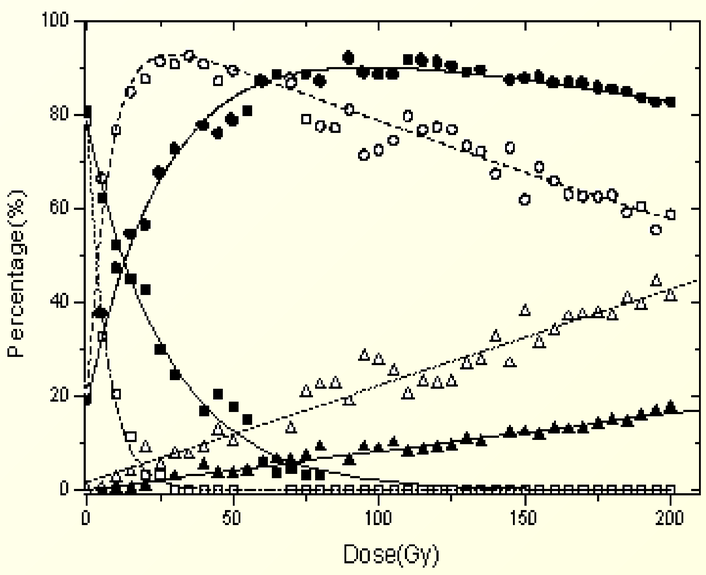


Figure 1. Fitting with Cowan's model of percents for the tree plasmid conformational forms for samples with 200 ng/μl (open symbols) and 500 ng/μl (closed symbols). Super coiled (□), Circular (○), Lineal (Δ).

| Sample | λ(hit/Gy) | ρ(cut/Gy) | b | Error(%) |
|---|---|---|---|---|
| 200 ng/μl | $13{,}01\times10^{-2}$ | $19{,}38\times10^{-4}$ | 0,00204 | 5,2 |
| 500 ng/μl | $3{,}90\times10^{-2}$ | $7{,}98\times10^{-4}$ | 0,00208 | 3,7 |

The Yield of SSB and the DSB directly produced can be find as:

$$G_{DSB}^{dir}[\mu mol/J]=\rho\cdot C_{DNA}[\mu mol\cdot dm^{-3}]/d(kg/dm^3)$$
$$G_{SSB}[\mu mol/J]=\lambda\cdot C_{DNA}[\mu mol\cdot dm^{-3}]/d(kg/dm^3)$$

The size of the zone where two SSB in opposite strand can produce a DSB (taboo zone) will be:

$$\Delta[pb]=b\cdot Length_{DNA}[pb]$$

And we get:

| Sample | $D_{37}$(Gy)=1/λ | $G_{SSB}(\mu mol/J)$ | $G_{DSB}^{dir}(\mu mol/J)$ | Δ(pb) |
|---|---|---|---|---|
| 200 ng/μl | 7,69 | $1{,}53\times10^{-2}$ | $2{,}11\times10^{-4}$ | 6,04 |
| 500 ng/μl | 25,64 | $1{,}06\times10^{-2}$ | $2{,}16\times10^{-4}$ | 6,16 |

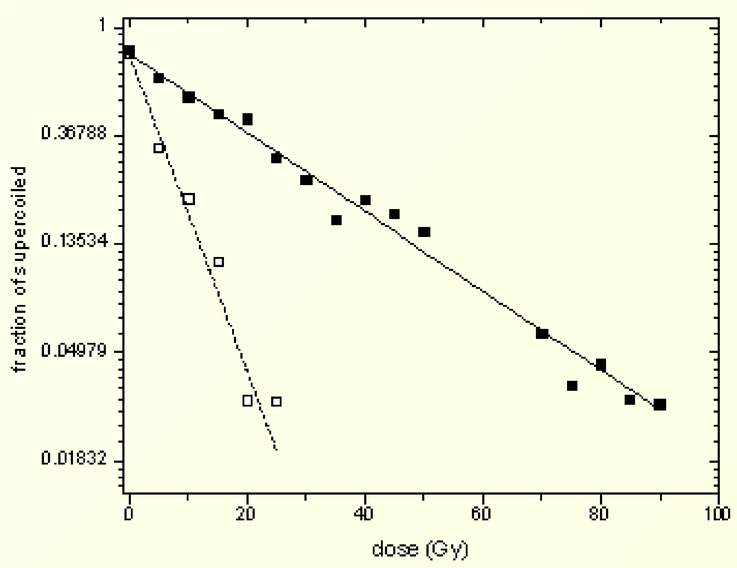


Figure 2. Yield of SSB as a function of Dose for γ- irradiation for sample with 200 ng/μl (open symbols) and 500 ng/μl (closed symbols).

### *Kinetics Model*

Combining elements of target theory of radiation damage and of homogeneous reaction kinetics and expression for the survival dose was obtained by Mark. (Mark et al 1989).

$$D_{37}=\frac{1}{\varepsilon\cdot g}\left\{c_{DNA}+\frac{1}{k_{DNA}}\sigma\right\}$$

were ε and efficiency factor, g is the mole number of OH*per volume per dose, $k_{DNA}$ is the rate constant of reaction of OH* with DNA and σ is the scavenging capacity (in our case was assumed to be zero). Is possible find the efficiency factor of OH* interaction with DNA as:

$$\varepsilon_{SSB}(OH^*)=\frac{c_{DNA}}{D_{37}\cdot g}$$

### *Calculation of $D_{37}$ and the G Value of SSB and DSB formation*

The $D_{37}$ was calculated from the reciprocal of the slope, figure 2. The concentration of SSBs and DSBs were calculated by

$$G(SSB)=\frac{C_{DNA}}{D_{37}\cdot\rho}\qquad G(DSB)=\frac{slope\cdot C_{DNA}}{\rho}$$

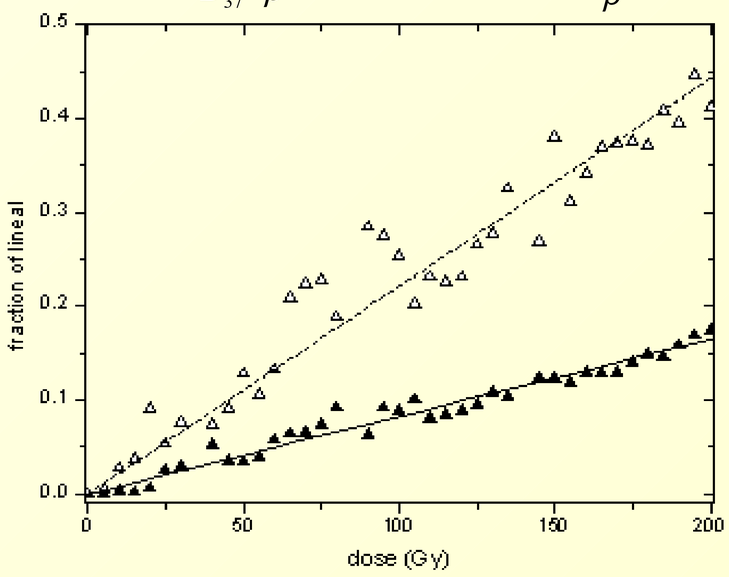


Figure 3. Yield of DSB as a function of Dose for γ- irradiation for sample with 200 ng/μl (open symbols) and 500 ng/μl (closed symbols).

| Concentration | $D_{37}$ [Gy] | G(SSB) [μmol J$^{-1}$] | G(DSB) [μmol J$^{-1}$] | $\varepsilon_{SSB}$(OH*) |
|---|---|---|---|---|
| 200 ng μl$^{-1}$ ($1{,}09\times10^{-7}$ mol dm$^{-3}$) | 6,72 | $1{,}6\times10^{-2}$ | $2{,}4\times10^{-2}$ | $5{,}7\times10^{-2}$ |
| 500 ng μl$^{-1}$ ($1{,}09\times10^{-7}$ mol dm$^{-3}$) | 27,28 | $1{,}0\times10^{-2}$ | $2{,}2\times10^{-2}$ | $3{,}5\times10^{-2}$ |

## Discussion

The values of λ and ρ obtained from the statistical model fit (table 1) agree with the ones reported by Spotheim-Maurizot $\lambda=K_S=(3.9\pm0.2)\times10^{-2}$ Gy$^{-1}$ y $\rho=K_d=(6.9\pm1)\times10^{-4}$ Gy$^{-1}$. The Yields of SSB and DSB for both DNA samples calculated by the Cowan's model are similar. The values of the Δ(pb), necessary number of base pairs for DSB production starting from tow SSB in opposite strands are similar.

The calculated efficiency factor of OH* interaction with DNA ($5{,}7\times10^{-2}$ y $3{,}5\times10^{-2}$) are in the order of the reported by Önal (Önal et al 1988), $7{,}8\times10^{-2}$ for a DNA concentration of $1.5\times10^{-7}$ mol dm$^{-3}$, similar to the one used in this work.

Other workers have irradiated aqueous DNA without scavenger (Milligan 1993, Hempel 1987, Schans 1978, Spotheim 1990). We compared also the Milligan results with ours, figure 4.

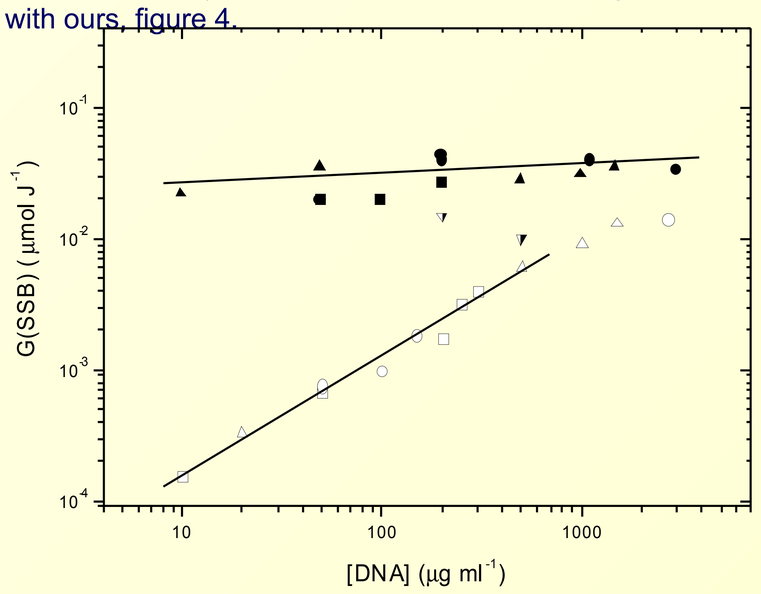


Figure 4. G(SSB) for pUC18(Δ), SV40(□), pEC(○) and at various concentration without scavengers(closed symbols) and the same in $10^{-3}$ mol dm$^{-3}$ DMSO (open symbols) (Milligan 1993), compared with the pBKS(▽) without scavengers irradiated by us.

The decrease in the values obtained could be by the fact that ours samples have only a 80% of purity and not the >95% as the Milligan's samples. However ours results of $G_{SSB}$ are in the order with the $3{,}5\times10^{-2}$ obtained by him.

## Conclusion

The results obtained in general agree with the results reported in similar studies, indicating that the obtained data and the experimental method developed are reliable. Irradiations in different conditions are planned to be accomplished in order to validate a theoretical model proposed by our group.

## Acknowledgements

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